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FIRST BIANNUAL TECHNICAL PROGRESS REPORT
C. WILSON, Grant # N00014-92-J-1113
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Introduction

I am submitting this report on the basis of my reading of Attachment Number 2 of the notice of award mailed to me in January, 1992. That attachment included no particular instructions on the format of this report, so I have taken the liberty of imposing a format of my own. I hope this is satisfactory.

The project was officially funded in October, 1991, although I did not receive notification until January 1992. Nonetheless, I make this report based upon the official anniversary date of the project.

Overall Summary of Activities

Since the project was funded we have purchased a Sun SPARCStation 2, which has arrived and been outfitted with the necessary software for carrying out the computer modeling studies. A search for an appropriate postdoctoral fellow has begun, but none has been identified so far. We have applied our personnel funds by employing one of the existing postdoctoral fellows in the laboratory part time on the project (he may become so interested as to convert completely to this project, which would be a satisfactory end to our search). In addition, we have employed a technician who has been assigned to the patch clamp studies funded as a part of the project.

The experimental studies, whose goal is to gain quantitative descriptions of ionic conductances of neostriatal spiny neurons suitable for use in a computer model of the neuron, are making rapid progress. We were able to bring this aspect of the project up to speed quickly by taking advantage of an already active research program on modulation of ionic conductances by the neuromodulators dopamine and acetylcholine. We currently have a full set of data on the kinetics and voltage sensitivity of 3 of the currents most important for the model (the fast sodium and the rapidly- and slowly-inactivating transient potassium current) and we are now collecting data on a set of high threshold calcium conductances (which are proving to be somewhat more complicated). It is fortunate that these data include a study of the modulation of the conductances, as this will allow us to include these long-term changes in neuronal characteristics in the model. There is a growing body of evidence to suggest that such modulation may allow neurons to alter their fundamental computational properties on the time scale of minutes or hours. The experimental work has yielded one paper submitted for publication, and work for an additional paper is finished and will be written soon.

The principal effort in the computational side of the project has been the incorporation of the experimental data into the neuron model and verifying that the behavior of the model matches that of the neurons as seen in voltage-clamp studies. Two channels have been fully incorporated and verified in this fashion. These are the slowly-inactivating and rapidly-inactivating potassium channels. Our initial simulations using these conductances in the model of the striatal spiny neurons suggests that the slowly-adapting conductance may confer upon the cell some computationally important properties. Most notable among these is a conditional synaptic cooperativity, in which the ability of nearby synapses to act cooperatively in transmitting their signals to the soma is dependent upon the history of prior synaptic input to the same region of the dendrite over a period of several seconds.

Detailed Description of Completed Work

Completed experimental work on the sodium channel and its modulation by dopamine has already been described in a paper submitted for publication. This work showed that the voltage-sensitivity of inactivation of sodium channels is specifically regulated by dopamine, acting on both D1-family and D2-family receptors. Because of the large dopamine innervation of neostriatal projection neurons and the demonstrated importance of dopamine in the function of the neostriatum, it has long been a mystery that dopamine does not appear to act as a conventional neurotransmitter in this region. Practically all models of neostriatal function continue to treat the dopaminergic input as a conventional (usually inhibitory) input that is integrated with other inputs in the classical fashion. Although there have been a number of physiological studies suggesting that this is not so, there has been no plausible counterproposal on the function of dopamine. We have presented a counterproposal. Our work indicates that a major effect (perhaps the principal effect) of dopamine is to reduce the availability of sodium channels and thus to alter the translation of synaptic potentials into trains of action potentials. In addition to this work we have increased our sample of biophysical data on the kinetics of voltage-activated potassium currents and have analyzed those data to generate a model of the Hodgkin-Huxley type for that current as it exists in neostriatal neurons. We have also extended our observations of these currents to the tissue slice preparation. Using some preliminary simulations of the potassium currents as a guide, we designed an experimental protocol to isolate the effect of the slowly-inactivating transient potassium current in current-clamp recordings from tissue slices. Intracellular recording experiments revealed this current, and we were able to measure several parameters, including the time constant of recovery from inactivation and the voltage sensitivity of inactivation, from current clamp recordings in slices at 35°C. This allowed us to confirm that the current was acting in the intact tissue in the same way as that seen in dissociated cells, and also gave a rough check on the termperature dependence, at least for recovery from inactivation (the time course of which we were able to measure fairly accurately in the slice). Recovery from inactivation is important, because it is the parameter that determines the residual effect of previous conditions of membrane potential. Its time course determines the span of membrane potential history to which the conductance is sensitive. McCormick has shown that recovery of inactivation of this same current in thalamic neurons is very fast (~100 ms), so that the cell rapidly forgets its history of inactivation when it returns to the hyperpolarized state. In striatal cells, however, the comparable ion channel recovers much more slowly (seconds). This may be related to the fundamental difference between the modes of firing of these cells. Thalamic cells acting at least part of the time as rhythmic pacemakers, while striatal cells almost never show oscillatory activity.

Computer simulations of the transient potassium currents currently underway have shown that the slowly-inactivating current interacts with the anomalous rectification to create three membrane potential ranges. At relatively polarized membrane potentials (-75 to -95 mV) corresponding to the hyperpolarized episodes in the spontaneous activity of the neurons, the membrane resistivity is very low (2000-3000 ohm-cm^2) owing to the strong activation of the anomalous rectifier. At membrane potentials near the action potential threshold (-45 to -55 mV) the membrane resistivity is again relatively low, owing to the slowly-inactivating potassium current. At membrane potentials between these, input resistivity becomes much higher (20000 to 50000 ohm-cm^2), as both of these currents are mostly (but not completely) deactivated. These results match measurements taken both *in vivo* and *in vitro* in neostriatal neurons. Even in the high resistivity range it does not appear that a voltage-independent leak conductance plays any

significant role in determining the overall input resistance of the neuron. This dependence of membrane resistivity on membrane potential alters the input resistance of the neuron, making it very sensitive to small perturbations when in the in-between range of membrane potentials and relatively stable when in the depolarized or hyperpolarized state. This is important in generating the quasi-bistable behavior of the neurons. But the dependence of membrane resistivity on membrane potential does much more than that. It adjusts the effective electrotonic length of the dendrites. When the membrane resistivity is high, the dendritic field becomes electrotonically compact (0.2 to 0.5 length constants) while at the two more stable membrane potential states the cell becomes much more extended (up to about 1.75 length constants at -90 mV). This means that synaptic inputs arising during the transition to the depolarized state are transiently very effective and the cell receives a boost into the depolarized state, from which further synaptic input may evoke action potentials. Once in the depolarized state, however, the cell becomes somewhat less sensitive to synaptic input again. It is important that this state occurs below the firing threshold, and so while in the depolarized state the cell is enabled for firing, but requires an additional level of convergent input to be sufficiently depolarized to fire. An additional twist to this is introduced by the fact that this is an inactivating current. If the cell is maintained in the depolarized state, or is repeatedly depolarized, it will become more sensitive over a period of seconds, as the limiting potassium current is slowly inactivated. Slow recovery from inactivation means that this could accumulate over a period of time when the cell is repeatedly depolarized and repolarized, making the cell more sensitive to repeated stimuli. We will be making quantitative predictions about this effect using the computer simulations, which we hope to test *in vivo*.

Plan for Upcoming 6 Months

Experimental work over the next 6 months will be directed at unraveling the kinetics of high threshold calcium currents in neostriatal spiny neurons. This task is complex because it is very difficult to experimentally distinguish between the existence of more than one species of channel with similar kinetics and complex kinetic behavior of a single channel. There is good reason to expect that both of these are contributing to the behavior of the calcium channels we are studying. The distinction is important, however. Under less constrained circumstances than those that arise in voltage clamp studies (e.g., free-running behavior of the cell *in vivo*), these apparently small differences produce very divergent effects on the neurons. This sort of thing is not surprising in non-linear systems. To avoid being led astray in this manner, our intention is to make biophysically accurate models of the ionic conductances, not simplifications that appear phenomenologically ok under conditions of voltage-clamp experiments. Thus we will be repeating our biophysical experiments using a variety of pharmacological treatments that have been shown to differentially affect the various species of calcium conductances. This is a controversial area, and none of these drug treatments are straightforward blockers in the sense that TTX is a blocker of sodium channels.

Some of the modeling in the next few months will be dedicated to testing ideas about the kinetics of the calcium channels. One useful function of the computer simulations is in the design of experiments to test candidate kinetic models of the channels. Simulating the models is useful for determining a set of experimental conditions that will distinguish between them, which can then be translated into a critical experiment. The remainder of the effort will go into simulating the effects of the transient potassium channels and their modulation on the integration of synaptic transmission in the neostriatal neuron model. This will be a continuation of the work done earlier using the inwardly-rectifying potassium channel, and in fact the inward rectifier will

be included in the simulations. This is because the results so far (as described above) indicate these this channels have their most interesting effects in their interactions with the inwardly rectifying channel.

Our modeling work on the transient potassium conductance has also made a prediction that can be tested *in vivo*. It predicts that by poisioning the voltage-sensitive potassium channels we could destroy the depolarized state as a state, and that synaptic inputs would produce much greater and more variable depolarizations (rather than a relatively constant depolarized plateau). We have begun to test this prediction *in vivo* using ion channel poisons introduced into the interior of neurons through the microelectrode. During the upcoming 6 months we will determine whether the quasi-stable membrane potential state seen in striatal neurons is really a ceiling effect produced by the potassium current.

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